

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
1 April 2004 (01.04.2004)

PCT

(10) International Publication Number
WO 2004/027065 A2

- (51) International Patent Classification⁷: C12N 15/53, 9/02, C12P 7/26
- (21) International Application Number: PCT/EP2003/010473
- (22) International Filing Date: 19 September 2003 (19.09.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 02021098.5 23 September 2002 (23.09.2002) EP
- (71) Applicant (*for all designated States except US*): DSM IP ASSETS B.V. [NL/NL]; Het Overloon 1, NL-6411 TE Heerlen (NL).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): KATAOKA, Michihiko [JP/JP]; 24-308 Okazaki-Irie-cho, Sakyo-ku, Kyoto-shi, Kyoto-fu, Kyoto 606-8322 (JP). SHIMIZU, Sakayu [JP/JP]; 6-9 Yamashita-cho, Tokiwa, Ukyo-ku, Kyoto-shi, Kyoto-fu, Kyoto 616-8212 (JP).
- (74) Agents: KELLER, Günter et al.; Lederer & Keller, Prinzregentenstrasse 16, 80538 München (DE).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: ENONE REDUCTASE GENE AND MICROBIAL PRODUCTION OF LEVODIONE

(57) **Abstract:** Disclosed is an isolated DNA comprising a nucleotide sequence coding for an enzyme having enone reductase activity wherein the enzyme is characterized by the following physico-chemical properties: (a) molecular mass: $61,300 \pm 5,000$ Da (estimated using gel filtration, consisting of one subunit); (b) co-factor: NADPH and NADH; (c) substrate specificity: active on α, β -unsaturated ketons; (d) optimum temperature: 55-60°C at pH 7.4; and (e) optimum pH: pH 4.5-8.5.

WO 2004/027065 A2